14P

NASA TECHNICAL TRANSLATION NASA TT F-15,720

LONG-TERM EFFECT OF HIGH VACUUM ON MICROORGANISMS

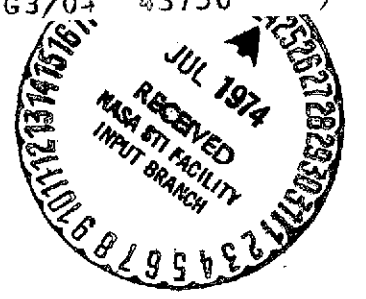
A. A. Imshenetskiy, C. B. Lysenko,
B. F. Udovenko and A. M. Bumenko

Translation of: "Dlitel'noye Vozdeystviye Glubokogo Vakuuma na Mikroorganizmy," Mikrobiologiya, Vol. 42, No. 5, pp. 836-838, 1973.

(NASA-TT-F-15720) LONG TERM EFFECT ON HIGH VACUUM ON MICROORGANISMS (Techtran Corp.) 7 p HC $4.00 CSCL 06H

N74-28564

Unclas
G3/04 43750



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D. C. 20546 JULY 1974

STANDARD TITLE PAGE

1. Report No. NASA TT F-15720	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle: Long-Term Effect of High Vacuum on Microorganisms		5. Report Date: July 1974 6. Performing Organization Code
7. Author(s): A. A. Imshenetskiy, C. B. Lysenko, B. F. Udovenko and A. M. Bumenko		8. Performing Organization Report No. 10. Work Unit No. 11. Contract or Grant No. NASw-2485
9. Performing Organization Name and Address: Techtran Corporation, P.O. Box 729, Glen Burnie, Maryland 21061		13. Type of Report and Period Covered: Translation
12. Sponsoring Agency Name and Address: National Aeronautics and Space Administration, Washington, D. C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of: "Dlitel'noye Vozdeystviye Glubokogo Vakuuma na Mikroorganizmy," Mikrobiologiya, Vol. 42, No. 5, pp. 836-838, 1973.

16. Abstract

The conidia of Aspergillus terreus, the spores of Bacillus megaterium and the vegetative cells of Sarcina flava were subjected to high vacuum. The combined action of high vacuum and low temperature for 500 hours failed to destroy these microorganisms.

17. Key Words (Selected by Author(s))	18. Distribution Statement: Unclassified-Unlimited		
19. Security Classif. (of this report): Unclassified	20. Security Classif. (of this page): Unclassified	21. No. of Pages: 17	22. Price: 4.00

NASA-HQ

LONG-TERM EFFECT OF HIGH VACUUM ON MICROORGANISMS

A. A. Imshenetskiy, C. B. Lysenko,
B. F. Udovenko and A. M. Bumenko

The problem of the biological effect of a high vacuum on microorganisms /836
is undoubtedly a both theoretical and practical interest.

At one time the opinion prevailed amongst scientists that the physical factors of outer space, ones such as high vacuum, extremely low temperatures, and shortwave ultraviolet rays, can in a short time inactivate viable germs of life taken into outer space. However, as exploration of outer space and the planets of the solar system has advanced, the need has arisen for subjecting all theoretical arguments to experimental verification. Of particular interests among the physical factors of outer space is the high vacuum, since it is permanently present in space, and in addition not enough study has been made of it from the biological viewpoint.

There are at the present time data in the literature indicating that application of a high vacuum for a short period (up to five hours) has no lethal effect on any type of microorganism (Imshenetskiy and co-workers, 1964); Imshenetskiy and Lysenko, 1970; Portner et al., 1961). As regards the protracted presence of microorganisms in a high vacuum, there is no unanimous opinion regarding the stability of microorganisms (Brueschke et al., 1961; Morelli et al., 1962; Cameron et al., 1970).

In this context studies were conducted in the present project to ascertain the survival rate of microorganisms after being subjected to the action of a high vacuum for 500 hours.

Procedure

The studies were conducted with conidia of the fungus *Aspergillus terreus*, vegetative cells of *Sarcina flava*, and spores of *Bacillus megaterium*. The cultures in question were grown on dense nutrient media (agar wash or MPA)

at 28°, and then washed with sterile tap water, and suspensions of the cultures in the amount of 0.1 ml were then applied to slides or paper filters. The latter were placed in sterile Petri dishes and held in a thermostat at 40° at 5 - 6 hours. After the water had evaporated the preparations were transferred to a high-vacuum chamber and exposed to a pressure of 10^{-9} mm Hg for 500 hours.

After the experiments had been completed the preparations were placed in flasks containing 10 ml sterile tap water and glass beads. On agitation of the flasks containing the preparations the glass beads separated the cells from the slides or disintegrated paper filters.

The initial microorganism suspensions contained in this manner were subsequently diluted with sterile tap water. The suspension of *Aspergillus terreus* conidia was sprinkled to the surface of the agar wash, and the suspensions of *Sarcina flava* and *Bacillus megaterium* into melted and cool MPA. The survival rate of the microorganisms was evaluated on the basis of the number of colonies growing in the nutrient media. Control preparations were stored in a refrigerator at normal atmospheric pressure and a temperature of -5°.

In addition, the survival rate in the case of the microorganisms indicated above was investigated 100 days after they had been removed from the test compartment. The experimental preparations, like the control ones, were stored at -5° and a pressure of 760 mm Hg. /837

Results

On the basis of the experiments conducted data were obtained characterizing the stability of the microorganisms after being subjected to the combined action of fluctuations in temperature and pressure (see Figure 1). Of the three culture studies the conidia of *Aspergillus terreus* possessed the greatest stability. For example, in the 10^{-2} and 10^{-3} suspension dilution rate of the conidia was respectively 114 and 98%. The highest ability of the conidia of this fungus was also established after a less protracted stay (72 hours) in a high vacuum (Imshenetskiy and Lysenko, 1970).

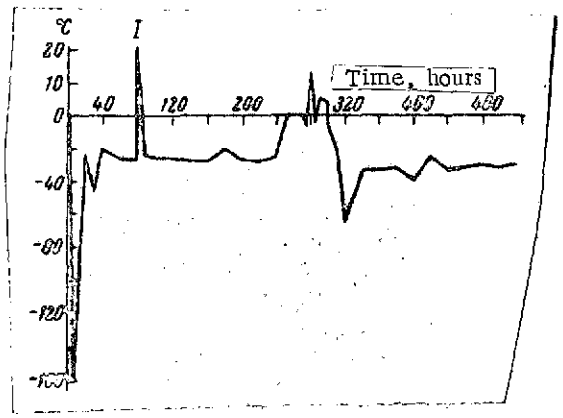


Figure 1. Variation in Temperature During Exposure of Preparations in a High Vacuum Equalling 10^{-9} mm Hg (in Position I the Pressure Was 10^{-3}mm Hg).

As has already been pointed out earlier (Imshenetskiy and Lysenko, 1970), the sperical forms of bacteria are characterized by much greater tolerance to a high vacuum then are the rodshaped ones. In particular it has been found that in the case of *Sarcina flava, Micrococcus letus and Micr. aurantiacus,* the amount of viable cells was more than 50% after a stay of 3 hours in a high vacuum. At the same time, the rodshaped forms *Pseudomanas byoceana, Ps. fluorescens, Escherichia coli,* and others perished almost in their entirely after exposure to vacuum.

A large number of viable cells of *Sarcina flava* remained after application of the action of 500 hours of a high vacuum. In the case of the 10^{-1} suspension dilution it amounted to 98%, and 87% in the case of the 10^{-2} dilution. It is to be noted in analysis of the tolerance of nonsporogenous *Sarcina flava* bacteria after protracted (500 hours) and brief (72 hours) stay in a high vacuum that the survival rate of the cells of this culture increased with increase in the time of exposure to the high vacuum.

A similar pattern was observed by Hagen and co-workers (Hagen et al., 1971), who studied *Micrococcus sp.* after this culture has been subjected to a deep vacuum and temperatures of +34, +59, and -105°.

As regards the tolerance of spores of *Bacillus megaterium*, it was just as high as that of the conidia of *Asp. terreus*. The amount of viable spores after application of a vacuum for a 500 hours was 112%, this indicating that they are better preserved in a high vacuum. This is approximately 35% less than after three hours of exposure of a vacuum (Imshenetskiy and Lysenko, 1970).

In comparing the survival rate of the microorganisms investigated which were exposed in a vacuum on paper filters and on glass slides, note must be made of the high survival rate of the spores and vegetative cells. However, /838 detailed analysis of the results obtained reveals that the paper filters foster better preservation of the spores and vegetative cells of the bacteria. In this particular case the filter paper apparently retains a certain amount of water during the stay of the preparations in the vacuum and thereby creates a certain microclimate for the microorganisms which differs from the conditions present on the glass slides. At the same time, the number of conidia of *Asp. terreus* present in the high vacuum on glass slides or on filter paper was the same.

Study of the control and experimental preparations of the conidia, spores, and vegetative cells of the bacteria stored after the experiment for 100 days at atmospheric pressure has a temperature of -5° revealed no substantial difference in their survival rate.

Thus the combined action of the high vacuum, low, extremely low, or room temperature on the spores and vegetative cells of bacteria and conidia of fungus does not result in their destruction. The microorganisms also exhibit a good toleration of variation from pressure 10^{-3} to 10^{-9} mm Hg.

BIBLIOGRAPHY

1. Imshenetskiy A. A., N. Bagrov and S. Lysenko, *Dokl. AN SSSR*, Vol. 154, p. 1188, 1964.
2. Imshenetskiy A. A. and S. V. Lysenko, in the book: "Zhizn' vne Zemli i Metody eye Obnaruzheniya," [Life Outside the Earth and Methods of Detecting it], "Nauka" Press, Moscow, p. 106, 1970.
3. Brueschke E., R. Suess and M. Willard, *Planetary Space Sci.*, Vol. 8, p. 30, 1961.
4. Cameron R. E., F. A. Morelli and H. P. Conrow, *JPL Technical report 32-1454*, Pasadena, California, March, 1970.
5. Hagen C. A., J. F. Godfren and H. R. Green, *Space Life Sciences*, Vol. 3, p. 108, 1971.
6. Morelli A., P. Fehlner and H. Stembridge, *Nature*, No. 4850, Vol. 196, p. 108, 1962.
7. Portner D. M., Spiner, D. R., R. K. Hoffman and C. R. Phillips, *Science*, Vol. 134, p. 2047, 1961.

Translated for the National Aeronautics and Space Administration under contract No. NASw-2485 by Techtran Corporation, P. O. Box 729, Glen Burnie, Maryland, 21061; translator: William L. Hutcheson.